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Clinical studies

Trace elements, oxidative stress and glycemic control in young people with type 1 diabetes mellitus

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ABSTRACT

Trace elements and oxidative stress are associated with glycemic control and diabetic complications in type 1 diabetes mellitus. In this study, we analyzed the levels of serum copper, zinc, superoxide dismutase (SOD) activity, and malondialdehyde (MDA) and urinary MDA and 8-hydroxy-2′-deoxyguanosine (8-OHdG) in 33 type 1 diabetic patients with optimal and suboptimal glycemic control ($HbA_{1C} < 9.0\%$) and 40 patients with poor glycemic control ($HbA_{1C} \geq 9\%$) and 27 age- and sex-matched non-diabetic controls to evaluate the differences between these markers in different glycemic control states. Diabetic patients, especially poor-glycemic-control subjects ($HbA_{1C} \geq 9\%$), exhibited significantly lower levels of serum zinc and increased levels of serum copper (and, therefore, increased serum copper-to-zinc ratios), serum SOD, blood MDA, and urinary MDA and 8-OHdG, relative to non-diabetic subjects. Furthermore, significant correlations existed in these patients between the serum copper, serum copper-to-zinc ratio, and urinary MDA (all $p < 0.001$) and the levels of urinary 8-OHdG ($p = 0.007$) and HbA_{1C}. Our results suggest that high serum copper levels and oxidative stress correlate with glycemic control. Therefore, strict glycemic control, decreased oxidative stress, and a lower copper concentration might prevent diabetic complications in patients with type 1 diabetes mellitus.

Introduction

Type 1 diabetes mellitus is a disorder resulting from autoimmune destruction of insulin-producing beta cells. Hyperglycemia is the crucial factor leading to diabetic micro- and macro-vascular complications. Several mechanisms have been proposed – including polyol and hexosamine pathways, protein kinase C activation, and the formation of advanced glycation end products (AGEs) – to explain how hyperglycemia leads to the chronic complications of diabetes mellitus [1–5]. The free radicals that form during the glucose autoxidation process will also oxidatively damage lipids, proteins, and nucleic acids, as well as cause other types of biological damage [1–5]. In addition, trace elements, particularly copper and zinc, are also involved in free radical reactions and diabetic cardiovascular complications [6,7].

Malondialdehyde (MDA) is a product of polyunsaturated fatty acid peroxidation [8]. Martín-Gallán et al. and Fathy et al. found that MDA was significantly elevated in young type 1 diabetic patients regardless of the presence of microangiopathy complication [9,10]. MDA excretion is also associated with sudomotor dysfunction in early type 1 diabetes mellitus [11]. When free radicals attack nuclear DNA, the repair process results in oxidized nucleosides and the base 8-hydroxy-2′-deoxyguanosine (8-OHdG), which are all excreted into urine [12,13]. Therefore, 8-OHdG can be monitored to serve as an indicator for DNA oxidation. Goodarzi and Hata et al. reported independently that increased urinary 8-OHdG occurs at the early stages of type 1 diabetes [2,14]. Significant increases in serum 8-OHdG in type 1 diabetes and urinary 8-OHdG in type 2 diabetes have also been noted previously [2,5,14].

Copper, zinc superoxide dismutase (Cu–Zn SOD) is an important antioxidant enzyme that protects cells from superoxide toxicity.

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Zinc and copper are two of the most important elements in the human body; they are involved in the metabolism of oxygen and in some redox reactions. Zinc serves as an antioxidant – it is an essential component of Cu–Zn SOD – and can stabilize thiols in zinc proteins, including metallothioneins and zinc-finger transcription factors [15]. Zinc deficiency may adversely affect immune responses and increase oxidative stress in many chronic diseases [6]. Copper displays pro-oxidant and antioxidant properties. It is also a component of Cu–Zn SOD, and it promotes the formation of reactive oxygen species via Haber–Weiss and Fenton-like reactions [4]. Excess copper will produce peroxidative damage to membrane lipids and is associated with cardiovascular events [7]. Lefebvre et al. and Jansen et al. reported that zinc flux in pancreatic beta cells is an important factor for the secretion of insulin [16,17]. A previous study revealed the impaired metabolism of copper and zinc in type 1 diabetes mellitus [18]. Martín-Gallán et al. reported that SOD activity was higher in the erythrocytes of type 1 diabetic patients, independent of the presence of microvascular complications [9]. In addition, Suys et al. found that erythrocyte Cu–Zn SOD activity was higher in diabetic subjects than in healthy controls [19].

The incidence of type 1 diabetes is very low in the populations from Asian countries [20]. To the best of our knowledge, few reports describe studies of oxidative stress and trace elements in type 1 diabetes in young Asian people. This present study was designed to investigate changes in oxidative stress with regard to four aspects – oxidative stress-related metals (copper, zinc), an antioxidant enzyme (SOD), a lipid peroxidation marker (MDA), and a DNA oxidative marker (8-OHdG) – in young type 1 diabetic patients having different glycemic control states.

Materials and methods

Study subjects

Seventy-three subjects with type 1 diabetes mellitus, aged 7–26 years, admitted to Kaohsiung Medical Hospital, were recruited in this study. Glycated hemoglobin (HbA_{1C}), a marker for the average blood glucose level over the past 2–3 months, was used to monitor the diabetic patients' glycemic control states. The patients were divided into two groups in terms of their HbA_{1C} levels according to the Global IDF/ISPAD guidelines for diabetes in childhood and adolescence [20]. The incidence of type 1 diabetes mellitus is very rare in Taiwan. Furthermore, for adolescents it is hard to control the level of HbA_{1C} within the expected target range ($HbA_{1C} < 7.5\%$) in clinical care. Therefore, the optimal-glycemic-control subjects ($HbA_{1C} < 7.5\%$) were combined with the suboptimal-glycemic-control subjects (HbA_{1C} 7.5–9.0%) into an "optimal and suboptimal glycemic control" group ($HbA_{1C} < 9.0\%$; $n = 33$; 17 males, 16 females); subjects with $HbA_{1C} \geq 9.0\%$ were assigned to the "poor glycemic control" group ($n = 40$; 18 males, 22 females). Twenty-seven subjects with matched sex, age, and body mass index (BMI) were included as non-diabetic controls. These non-diabetic controls were selected from subjects who had visited our hospital for routine inoculations. All control subjects were checked by doctors and exhibited no systemic diseases. None of these subjects had a habit of cigarette smoking or alcohol consumption. This study was approved by the Institutional Review Board of Kaohsiung Municipal Hsiao-kang Hospital (IRB no: A94120638); written informed consent was obtained from all participants or their parents or guardians.

Sample preparation

Urine and blood samples were obtained from study subjects in the morning after an overnight fast. Part of the blood sample was centrifuged to obtain serum. Serum samples were stored at −70 °C until required for the analysis of copper, zinc, SOD, and MDA; the remaining sample was used to determine the value of HbA_{1C} using an NGSP-approved method based on high-performance liquid chromatography (HPLC). The urine samples were centrifuged to obtain the supernatants, which were also stored at −70 °C until required for analysis of MDA and 8-OHdG.

Measurement of copper, zinc, and SOD

The serum samples used for determining copper and zinc were suitably diluted with 2% HNO_3 and analyzed using a flame-atomic absorption spectrometer (PerkinElmer Model 5100 PC, Norwalk, CT, USA) at wavelengths 324.8 and 213.9 nm, respectively. Each sample was analyzed in duplicate. Quality control was performed strictly using standard reference materials (Seronorm™ Trace Elements Serum; Nycomed AS). The serum SOD activity was measured using an SOD Assay Kit (Sigma, Buchs, Switzerland). This assay employed Dojindo's highly water-soluble tetrazolium salt 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-*H*-tetrazolium (monosodium salt) (WST-1) to produce a water-soluble formazan dye upon reduction with a superoxide anion. The superoxide radical ions, generated from the conversion of xanthine to uric acid and H_2O_2 by xanthine oxidase (XO), converted WST-1 to WST-1 formazan, which absorbs light at 450 nm. SODs decrease the concentrations of superoxide ions and, thereby, decrease the rate of formation of WST-1 formazan.

Measurement of MDA

MDA was measured through HPLC using a C-18 column (JASCO Model 980-PU, Tokyo, Japan). The sample containing MDA was hydrolyzed with phosphoric acid in boiling water and reacted with thiobarbituric acid reactive substance (TBARS) to form MDA-TBA adducts. The mobile phase, methanol and potassium phosphate buffer (9:11), was delivered at a flow rate of 1.2 mL/min. A UV detector (JASCO UV-2075 Plus, Tokyo, Japan) was used for detection of these adducts at a wavelength of 532 nm.

Measurement of 8-OHdG

Urinary 8-OHdG was measured using an HPLC/tandem mass spectrometry (MS/MS) method [21]. The HPLC system consisted of a PE 200 autosampler, two PE 200 micropumps (PerkinElmer, Norwalk, CT, USA), and a Polyamine-II endcapped HPLC column (150 × 2.0 mm, 5 mm, YMC) equipped with an identical guard column (10 × 2 mm, YMC). The mobile phase was 80% acetonitrile containing 0.1% formic acid, delivered at a flow rate of 300 μL/min. The eluent of the HPLC system was connected to a triple-quadrupole mass spectrometer (API 3000, Applied Biosystems, Foster City, CA, USA) equipped with a TurboionSpray TM source. Electrospray ionization was performed in the positive mode. For all samples, the $[M+H]^+$ ion was selected by the first mass filter. After collisional activation, the $[M+H-116]^+$ ions, corresponding to BH_2^+, were selected by the last mass filter. Nitrogen was used as the nebulizing, curtain, heater (6 L/min), and collision gas. The Turboion-Spray™ probe temperature was set at 300 °C. The internal standard was $^{15}N5$-8-OHdG.

Statistical analysis

Data are expressed as mean ± standard deviation (SD). One-way ANOVA tests, used for continuous variables adjusted for age, gender, and BMI, were applied to determine the differences among the poor-glycemic-control patients ($HbA_{1C} \geq 9\%$), optimal-and-suboptimal-glycemic-control subjects ($HbA_{1C} < 9\%$),